

# Screening of key candidate genes and pathways for osteocytes involved in the differential response to different types of mechanical stimulation using a bioinformatics analysis

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**[Objective]** This study aimed to predict the key genes and pathways that are activated when different types of mechanical loading are applied to osteocytes.

**[Method]** The mRNA expression datasets (series number of GSE62128 and GSE42874) were obtained from Gene Expression Omnibus database (GEO). High gravity-treated osteocytic MLO-Y4 cell-line samples from GSE62128 (Set1), and fluid flow-treated MLO-Y4 samples from GSE42874 (Set2) were employed. After identification of the differentially expressed genes (DEGs), functional enrichment were performed. The common DEGs between Set1 and Set2 were considered as the key DEGs, then a protein-protein interactions (PPI) network was constructed using the minimal nodes from all the DEGs of Set1 and Set2, which linked most of the key DEGs. The exon expression of the key DEGs were also analysed by probe level data of Set1 and additional RNA-sequence data GSE70667 (two hours after fluid flow). Several open source softwares were employed to processes and analysis the original data. The bioinformatic results and its biological meaning were tested by *in vitro* and *in vivo* experiments.

**[Results]** The hypoxia related biological process and signaling pathway were the common functional enrichment terms among the DEGs from Set1, Set2 and PPI network. Regulation of circadian rhythm was an unexpected enrichment term in the DEGs from Set2. Both GSE70667 and Set1 showed alternative splicing of *Eno2* and *Mxi1*. The in vitro experiments showed the mechano-sensitive expression fashion of the common DEGs, and the expression change of clock genes under compressive force in MLO-Y4. A numerical simulation was built to show a possible mechanism that how the circadian rhythm regulate the spatial distribution in alveolar bone, which now is testing by *in vivo* experiments.

